

Amendments to the Claims:

This listing of claims will replace all prior versions, and listing of claims in the application.

1-94. (canceled)

Sub
examiner's
amend

1 95. (currently amended) A polynucleotide encoding a chimeric phosphorylation indicator having portions comprising a phosphorylatable domain and a fluorescent molecule, where the chimeric phosphorylation indicator comprises, in operative linkage, a donor molecule, a phosphorylatable domain, a phosphoaminoacid binding domain, and an acceptor molecule, at least one of said donor molecule and said acceptor molecule comprising a ~~non-oligomerizing~~ fluorescent molecule that has a reduced tendency to oligomerize compared with a corresponding naturally occurring fluorescent molecule,

wherein said donor molecule comprises ECFP (amino acids 1-227 of SEQ ID NO:6) or a substantially similar variant thereof having at least 80% amino acid sequence identity with ECFP (amino acids 1-227 of SEQ ID NO:6), said phosphorylatable domain comprises EEEAEYMNMAPQS (SEQ ID NO:23), said phosphoaminoacid binding domain comprises a Src homology domain-2, and said acceptor molecule comprises citrine (YFP; SEQ ID NO:10 having Q69M) or a substantially similar variant thereof having at least 80% amino acid sequence identity with citrine (YFP; SEQ ID NO: 10 having Q69M), wherein the amino acid following the initiating methionine is assigned the '1 position in the numbering of said donor and acceptor amino acid sequences,

wherein at least one of the donor molecule and the acceptor molecule comprises a mutation of an amino acid residue which corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO:6 or SEQ ID NO:10.

96-108. (canceled)

2 ~~109~~. (previously presented) The polynucleotide of claim ~~95~~¹, wherein the donor molecule and the acceptor molecule exhibit a detectable resonance energy transfer when the donor is excited.

110-114. (canceled)

~~15~~ ~~115~~. (previously presented) The polynucleotide of claim ~~147~~¹⁴, wherein the chimeric phosphorylation indicator further comprises a cell compartmentalization domain.

~~16~~ ~~116~~. (previously presented) The polynucleotide of claim ~~115~~¹⁵, wherein the cell compartmentalization domain is a membrane translocating domain.

~~17~~ ~~117~~. (previously presented) The polynucleotide of claim ~~116~~¹⁶, wherein the membrane translocating domain comprises an amino acid sequence CRQIKWFNRRMKWKK (SEQ ID NO:18).

~~18~~ ~~118~~. (currently amended) The polynucleotide of claim ~~116~~¹⁶, wherein the membrane translocating domain is operatively linked to the fluorescent protein through an amino acid sequence CCXXCC (SEQ ID NO:17) where X is any amino acid.

3 ~~119~~. (previously presented) The polynucleotide of claim ~~95~~¹, wherein the donor and the acceptor molecule exhibit a detectable resonance energy transfer when the donor releases energy, and the detectable resonance energy transfer is fluorescence resonance energy transfer.

120. (canceled)

~~19~~ ~~121~~. (previously presented) The polynucleotide of claim ~~147~~¹⁴, wherein the phosphorylatable polypeptide comprises a serine/threonine kinase phosphorylatable domain.

122-124. (canceled)

14
20 125. (previously presented) The polynucleotide of claim 147, wherein the phosphorylatable polypeptide comprises a tyrosine kinase phosphorylatable domain.

126-127. (canceled)

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21 128. (previously presented) The polynucleotide of claim 125 encoding a polypeptide comprising, in an orientation from the amino terminus to carboxy terminus, an ECFP (1-227) (amino acids 1 to 227 of SEQ ID NO:6) molecule, an SH2 phosphoaminoacid binding domain from Shc, a GSHSGSGKP (SEQ ED NO:22) linker, a phosphorylatable domain comprising EEEAEYMNMAPQS (SEQ ID NO:23), and citrine.

129. (canceled)

1
4 130. (previously presented) The polynucleotide of claim 95, wherein at least one amino acid of the phosphorylatable domain is phosphorylated.

4
5 131. (previously presented) The polynucleotide of claim 130, wherein the amino acid is serine, threonine, tyrosine, or a combination thereof.

132-144. (canceled)

1
6 145. (previously presented) The polynucleotide of claim 95, which is operatively linked to an expression control sequence.

6
7 146. (previously presented) The polynucleotide of claim 145, wherein the expression control sequence is a transcription regulatory element, a translation regulatory element, or a combination thereof.

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14 147. (currently amended) A polynucleotide encoding a chimeric phosphorylation indicator having portions comprising a phosphorylatable polypeptide and a fluorescent protein which polynucleotide is operatively linked to an expression control sequence, wherein said fluorescent protein comprises a non-oligomerizing

fluorescent protein having has a reduced tendency to oligomerize compared with a corresponding naturally occurring fluorescent protein, and wherein said fluorescent protein comprises a green fluorescent protein (GFP) or a fluorescent protein related to a GFP and wherein said fluorescent protein comprises a mutation which corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation.

14
22 ~~148~~. (currently amended) The polynucleotide of claim ~~147~~, wherein the expression control sequence is a transcription regulatory element, a translation regulatory element, or a combination thereof.

8 ~~149~~. (previously presented) A kit comprising at least one polynucleotide of claim ~~95~~.

8
9 ~~150~~. (previously presented) The kit of claim ~~149~~, comprising a plurality of polynucleotides encoding a plurality of chimeric phosphorylation indicators comprising at least two chimeric phosphorylation indicators differing from each other in at least one portion selected from the portions consisting of donor molecule, phosphorylatable domain, phosphoaminoacid binding domain, and acceptor molecule.

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amend. / 10 ~~151~~. (previously presented) The kit of claim ~~150~~, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise different phosphorylatable domains.

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amend. / 11 ~~152~~. (previously presented) The kit of claim ~~150~~, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise different donor molecules or acceptor molecules or both.

23 ~~153~~. (previously presented) A kit comprising at least one polynucleotide of claim ~~147~~. 14

²³
24 ~~154~~. (currently amended) The kit of claim ~~153~~, comprising a plurality of polynucleotides encoding a plurality of chimeric phosphorylation indicators comprising at least two chimeric phosphorylation indicators differing from each other in at least one portion selected from the portions consisting of phosphorylatable polypeptide and ~~non-~~oligomerizing fluorescent protein.

²⁴
25 ~~155~~. (previously presented) The kit of claim ~~154~~, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise different phosphorylatable polypeptides.

²⁴
26 ~~156~~. (currently amended) The kit of claim ~~154~~, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise different ~~non-~~oligomerizing fluorescent proteins.

²⁴
27 ~~157~~. (currently amended) A kit comprising at least one polynucleotide of claim ~~147~~,¹⁴ which polynucleotide is operatively linked to an expression control sequence, wherein the chimeric phosphorylation indicator further comprises a phosphoamino acid binding domain operatively linked to the phosphorylatable polypeptide, wherein the ~~non-~~oligomerizing fluorescent protein comprises an N-terminal portion and a C-terminal portion, and wherein the phosphorylatable polypeptide and operatively linked phosphoaminoacid binding domain is operatively inserted between the N-terminal portion and C-terminal portion of the ~~non-~~oligomerizing fluorescent protein.

²⁷
28 ~~158~~. (currently amended) The kit of claim ~~157~~, comprising a plurality of chimeric phosphorylation indicators comprising at least two chimeric phosphorylation indicators differing from each other in at least one portion selected from the portions consisting of phosphorylatable polypeptide and ~~non-~~oligomerizing fluorescent protein.

²⁸
29 ~~159~~. (previously presented) The kit of claim ~~158~~, wherein the chimeric phosphorylation indicators comprise different phosphorylatable polypeptides.

²⁷
30 ~~160~~. (currently amended) The kit of claim ~~157~~, wherein the plurality of different chimeric phosphorylation indicators comprise different non-oligomerizing fluorescent proteins.

12 ~~161~~. (previously presented) A vector comprising the polynucleotide of claim ¹~~95~~.

13 ~~162~~. (previously presented) The vector of claim ¹²~~161~~ which is an expression vector.

163-166. (canceled)

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Jim Fox on November 9, 2004.

The claims have been amended as follows:

~~95.~~ **An isolated** polynucleotide encoding a chimeric phosphorylation indicator [~~having portions comprising a phosphorylatable domain and a fluorescent molecule, where the chimeric phosphorylation indicator comprises, in operative linkage, a donor molecule, a phosphorylatable domain, a phosphoaminoacid binding domain, and an acceptor molecule, at least one of said donor molecule and said acceptor molecule comprising a fluorescent molecule that has a reduced tendency to oligomerize compared with a corresponding naturally occurring fluorescent molecule;~~] **comprising in operative linkage:**

~~[wherein said donor molecule comprises ECFP (amino acids 1-227 of SEQ ID NO: 6) or a substantially similar variant thereof having at least 80% amino acid sequence identity with ECFP (amino acids 1-227 of SEQ ID NO: 6);]~~ **a donor molecule comprising an amino acid sequence having at least 80% sequence identity to ECFP (amino acids 1-227 of SEQ ID NO: 6);**

~~[said] a~~ phosphorylatable domain [~~comprises]~~ **comprising** EEEAEYMNMAPQS (SEQ ID NO: 23);

~~[said] a~~ phosphoaminoacid binding domain [~~comprises]~~ **comprising** a Src homology domain-2; and

~~[said] an~~ acceptor molecule [~~comprises]~~ **comprising** [~~citrine (YFP; SEQ ID NO: 10 having Q69M) or a substantially similar variant thereof having at least 80% amino acid sequence identity with citrine (YFP; SEQ ID NO: 10 having Q69M)]~~ **an amino acid sequence having at**

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least 80% sequence identity to YFP (SEQ ID NO: 10), wherein the acceptor molecule has the mutation K69M with respect to SEQ ID NO: 10,

and wherein the amino acid following the initiating methionine is assigned the '1 position in the numbering of said donor and acceptor amino acid sequences, **and** wherein at least one of the donor or acceptor molecules comprises a mutation [~~of an amino acid residue~~] which corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO: 6 or [SEQ ID NO:] 10, **wherein said mutation results in a reduced tendency to oligomerize.**

14 ~~147~~ ⁹ 147. An **isolated** polynucleotide encoding a chimeric phosphorylation indicator [~~having portions comprising~~], **said indicator comprising:**

a phosphorylatable polypeptide; and

a fluorescent protein [~~which polynucleotide is operatively linked to an expression control sequence, wherein said fluorescent protein has a reduced tendency to oligomerize compared with a corresponding naturally occurring fluorescent protein, and wherein said fluorescent protein comprises a green fluorescent protein (GFP) or a fluorescent protein related to a GFP and wherein said fluorescent protein comprises~~] **comprising** a mutation which corresponds to an A206K mutation, an L221K mutation, an F223R mutation or an L221K and F223R mutation **of SEQ ID NOS: 6 or 10, wherein the amino acid following the initiating methionine is assigned the '1 position in the numbering of SEQ ID NOS: 6 and 10, or an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO: 2, wherein said mutation results in a reduced tendency to oligomerize,**

wherein said polynucleotide is operatively linked to an expression control sequence.

10 ~~151~~ ⁹ 150. The kit of claim ~~150~~ ⁹, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise [~~different~~] phosphorylatable domains **different from each other.**

11 ~~152~~ ⁹ 150. The kit of claim ~~150~~ ⁹, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise [~~different~~] donor molecules **different from each other** or acceptor

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molecules **different from each other** or both **donor and acceptor molecules different from each other**.

Support for the amendment regarding K69M of claim 95 can be found on p. 4, paragraph [0011], p. 18, paragraphs [0056] and [0057]. Support for the amendment regarding the numbering starting at the '1 position following the initiator methionine can be found in U.S. Patent 6,150,176 which is incorporated by reference on p. 38, paragraph [0099] and in Tsien *et al.*, *Ann. Rev. Biochemistry* 67: 509-544, 1998, which is incorporated by reference on p. 19, paragraph [0058].

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Rachel K. Hunnicutt whose telephone number is (571) 272-0886. The examiner can normally be reached on Mon-Fri 8:30 am - 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brenda Brumback can be reached on (571) 272-0961. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

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RKH

11/12/04